1. SCIENTIFIC ABSTRACT

The incidence of malignant melanoma is increasing more rapidly than any other type of cancer in the United States. Although often curable in the early stages, once the disease has spread long-term survival is unlikely. Furthermore, patients with melanoma are often young and there has been minimal response to standard treatment approaches, such as chemotherapy or radiation therapy. The use of immunotherapy to stimulate immune responses against melanoma cells has been successful in selected patients and has been reported to induce durable clinical responses in some patients with metastatic disease. The isolation of melanoma tumor antigens capable of specific T-cell recognition over the past decade has led to increased enthusiasm for the development of vaccines for the immunotherapy of melanoma.

Activation of T-cell responses depend on delivery of two signals, one antigen-dependent and the other antigen-independent. T-cells recognize antigens through the surface T-cell receptor only after processing into smaller peptide fragments and presentation by the MHC complex on antigen-presenting cells. This interaction represents the antigen-dependent signal and several candidate melanoma antigens have been identified that bind to the MHC class I and class II complexes for T-cell recognition. The optimal antigen(s) and their peptide derivatives are not known. However, their therapeutic potential is evidenced by a recent clinical trial using a modified gp100 peptide vaccine and interleukin-2 in HLA-A2 metastatic melanoma patients. This study found a 42% clinical response rate and will be evaluated in a larger randomized phase III trial in the near future.

The second signal required for T-cell activation is delivered by co-stimulatory molecules found on the surface of antigen-presenting cells. The best characterized of these molecules is the B7.1 molecule that becomes over-expressed on activated antigen-presenting cells. The B7.1 binds to ligands on the surface of T-cells and leads to stimulation (proliferation and cytokine production) or anergy depending on the nature of the T-cell surface ligand. When B7.1 binds to CD28 in the presence of an associated peptide-MHC interaction with a T-cell receptor the T-cell is activated into responding to the resented antigenic epitope. Furthermore, tumor cells may escape immune detection because they do not express sufficient levels of so-stimulatory molecules. Thus, potential tumor-reactive T-cells are made unresponsive when they enter the tumor microenvironment. Vaccine strategies have developed to include the addition of co-stimulatory molecules to improve the effectiveness of immunization. The optimal method for enhancing vaccines with co-stimulation is not known.

One strategy is to directly introduce the co-stimulatory molecule into viable tumor cells to improve their detection by circulating, or infiltrating T-lymphocytes. In vitro recognition of tumor cells by T-lymphocytes can be increased by transduction of co-stimulatory molecules or MHC molecules. T-cell responses were greatest when both molecules were present, but the responses were significantly better with the addition of co-stimulatory molecules compared to MHC molecules. This approach has the added advantage that specific tumor antigens are not needed since the transduced tumor cell

already presents its own repertoire of unique antigens. Thus, this approach can be applied to every patient regardless of the type and extent of antigen presentation.

We have recently tested this approach using a recombinant vaccinia virus expressing the human B7.1 co-stimulatory molecule gene in patients with accessible metastatic melanoma lesions. To date, we have treated eight patients with minimal vaccine-related toxicity and have one patient with a partial response and one patient without disease after surgical resection of residual tumor. Pre-clinical evidence has shown that the addition of multiple co-stimulatory molecules to poxvirus vectors can further enhance the ability of these vaccine to induce T-cell responses *in vitro* and *in vivo.* A vaccinia virus expressing human B7.1, ICAM-1, and LFA-3 was generated (designated rV-TRICOM), tested *in vitro* and in mice, and is now available for clinical application. This trial proposes to evaluate the effects of direct intra-lesional injection of rV-TRICOM into accessible cutaneous, sub-cutaneous, or lymph node melanoma lesions. We plan to determine the effects on injected lesions as well as the development of systemic anti-melanoma immunity that may lead to rejection of other distant melanoma lesions.